sensitization by Ca^{2+}. However, the molecular mechanism of the MCU's regulation by Ca^{2+} remains unclear. Recently, MICU1 has been identified as an EF-hand-containing regulatory component of the MCU. We set out to elucidate the role of MICU1 in the Ca^{2+} regulation of the MCU and to examine its possible contribution to the sensitization. Mitochondrial Ca2+ uptake was evaluated fluorometrically in suspensions of permeabilized MICU1 knockdown (MICU1KD) and control HeLa cells as ruthenium red-sensitive Ca^{2+} clearance from the incubation buffer. Clearance of small Ca^{2+} pulses elevating the $[Ca^{2+}]$ to <1uM was rapid in MICU1KD cells, whereas it was negligible in control cells. Large Ca^{2+} pulses increasing buffer $[Ca^{2+}]$ to >10uM were taken up effectively by both MICU1KD and control cells. The dose-response for the clearance of added Ca^{2+} was sigmoidal in the control cells. This was leftward-shifted and showed lesser cooperativity in the MICU1KD cells. MICU1KD rescue with wild type MICU1 restored the control type dose-response, whereas an EF-hand-mutant MICU1 shifted it to the right. When the time-dependent sensitization of the MCU was tested by a two-pulse protocol, the MICU1KD failed to show a further increase in Ca^{2+} sensitivity. As to the functional significance of altered Ca^{2+} handling in MICU1KD, these cells displayed lesser Ca^{2+} tolerance and more cell death under stress conditions. We propose that the MICU1, through its EF-hands, controls the $[Ca^{2+}]$ set-point for MCU channel closure. MICU1 helps mitochondria to respond to physiological $[Ca^{2+}]$ oscillations and provides some protection from Ca^{2+} overloading.

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Changes in Mitochondrial Oxygen Consumption can Associate with Increases in Total Mitochondrial Calcium

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Several novel candidate proteins have recently been proposed to mediate Ca^{2+} transport across the mitochondrial inner membrane. In addition to elucidating the basic mitochondrial Ca^{2+} handling machinery, these discoveries may uncover unknown modes of regulation by Ca^{2+} of mitochondrial processes. During the course of our studies into the function of the newly identified candidate protein, MICU1, we were intrigued to find that intact MICU1 knockdown HeLa cells increased their oxygen consumption by ~15% during store depletion-induced Ca^{2+} entry without any detectable rise in matrix free Ca^{2+}. There was however a robust increase in total mitochondrial Ca^{2+}, indicating effective buffering of the mitochondrial Ca^{2+} in knockdown cells. The oxygen consumption was then measured in both control and MICU1 knockdown HeLa cells at various $[Ca^{2+}]$, which caused a dose-dependent Ca^{2+} entry. At the lowest $[Ca^{2+}]$ (0.2mM $CaCl_2$), the rise in oxygen consumption in knockdown cells exceeded that in control cells which showed an increase matrix $[Ca^{2+}]$. With 1mM $CaCl_2$ addition, the rise in oxygen consumption was similar in knockdown and control cells (~30%). In control cells, oxygen consumption continued to rise with 10mM $CaCl_2$ addition, whereas knockdown cells showed a tendency for a lower oxygen consumption response relative to that with 1mM $CaCl_2$, suggesting dysfunction. For all Ca^{2+} concentrations, the oxygen consumption increases were entirely antimycin-sensitive, indicating a mitochondrial origin. Ca^{2+} addition to permeabilized knockdown cells triggered a ruthenium red-sensitive rise in oxygen consumption, confirming the mitochondrial origin and suggesting the lack of dependence on cytosolic and plasma membrane Ca^{2+} handling processes. Thus, mitochondrial Ca^{2+} influx by itself can cause stimulation of respiration. Alternatively, mitochondrial Ca^{2+} may be available to interact with Ca^{2+}-sensitive proteins prior to binding to buffering species.

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Sodium-Induced Calcium Flux via Mitochondrial NCE is Dissimilar when Derived from Matrix and Extra-Matrix Calcium Ion Concentrations

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Free Ca^{2+} plays a pivotal role in regulating cellular and mitochondrial processes in cardiomyocytes. Effective regulation of mitochondrial Ca^{2+} (mCa^{2+}) is vital for mitochondrial bioenergetics. Altered mCa^{2+} homeostasis resulting from cytosolic Ca^{2+} overload can lead to mitochondrial and cellular injury. mCa^{2+} dynamics are regulated primarily by influx via the mitochondrial Ca^{2+} uniporter (mCU) and efflux via the mitochondrial Na^+/Ca^{2+} exchanger (mNCE). Ca^{2+} flux across the inner mitochondrial membrane is modulated by the $m\Delta\Psi$ and $m\Delta pH$ and by the Ca^{2+} and Na^+ ion gradients. How the mNCE dynamically regulates mCa^{2+} remains to be elucidated. Previous attempts to characterize mNCE kinetics were limited in scope, because they focused on monitoring changes only on extra-matrix $[Ca^{2+}]$ or matrix $[Ca^{2+}]$ statically or under limited Ca^{2+} loading conditions. Here we explored in detail the kinetics of mNCE in energized mitochondria isolated from guinea pig hearts. We first added $CaCl_2$ (no buffer Na^+) to increase extra-matrix $[Ca^{2+}]$, and observed dynamic time-dependent changes in extra-matrix $[Ca^{2+}]$ and matrix $[Ca^{2+}]$, measured by indo-1 PP and indo-1 AM fluorescence, respectively. Then the mCU was blocked by ruthenium red and mCa^{2+} efflux was induced by adding increasing extra-matrix amounts of NaCl. We observed that the kinetics of mNCE depend both on the preceding uptake of Ca^{2+} and on the subsequent uptake of extra-matrix Na^+. Moreover, we found that Ca^{2+} efflux rates were dissimilar when derived from measurements of matrix $[Ca^{2+}]$ vs. extra-matrix $[Ca^{2+}]$, suggesting differential Ca^{2+} buffering. Thus, the amount and buffering of mCa^{2+} are crucial factors in the dynamics of Ca^{2+} efflux by mNCE. Our approach should yield novel findings in understanding the dynamics of Ca^{2+} efflux by mNCE.

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Modeling the Paradoxical Increase in Mitochondrial Calcium Buffering Power as Matrix Calcium Increases

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Mitochondria possess the remarkable ability to take up a massive, but finite, amount of Ca^{2+} before their primary function is compromised. Surprisingly, there has been very little progress in mathematically characterizing this phenomenon. We propose here a novel approach that implicitly models the known matrix Ca^{2+} buffering proteins and resultant Ca^{2+} sequestration in order to explain our recent experimental data from isolated guinea pig mitochondria. The model consists of our corroborated models of the TCA cycle and oxidative phosphorylation integrated with our previous models of the Na^+/Ca^{2+} cycle, a unique model of the Ca^{2+} uniporter coupled to the putative rapid-mode of Ca^{2+} uptake, and a Ca^{2+}-matrix buffering system. The model reproduces both the mitochondrial matrix and the extra-mitochondrial $[Ca^{2+}]$ dynamics observed when a bolus of Ca^{2+} is administered to the mitochondria followed by a bolus of Na^+. These results help elucidate why the reported change in matrix $[Ca^{2+}]$ of Ca^{2+}-loaded mitochondria appears significantly mitigated relative to the corresponding extra-mitochondrial $[Ca^{2+}]$ dynamics when Ca^{2+} efflux is initiated. Future work entails using the model to propose novel experiments in order to dissect the current model into components that explicitly describes these phenomena in a biophysically detailed manner. With the mitochondrial Ca^{2+}-sequestration system mathematically defined, computer simulations can then be used to design innovative therapeutics aimed at addressing the myriad of complications that arise due to cytosolic Ca^{2+} overload.

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Physiological Uncoupling of Mitocondria from Different Yeast Species

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A slow rate of oxygen consumption and a high transmembrane potential increase the rate of reactive oxygen species production (ROS). ROS in turn covalently attach and destroy membranes, proteins and carbohydrates. In order to avoid ROS-mediated damage, mitocondria contain diverse uncoupling mechanisms which accelerate the respiratory chain and lower the transmembrane potential. Two uncoupling mechanisms have been proposed. There are proteins that dissipate proton gradients either specifically (uncoupling proteins) or unspecifically (channels). A second mechanism is the activation of redox proteins that do not contribute to the proton gradient and therefore are sensitive to the ΔpH. In addition, pumps may suffer intrinsic uncoupling (slipping). In different yeast species, a number of mitocondrial uncoupling systems has been identified. Saccharomyces cerevisiae and Debaryomyces hansenii express highly regulated mitocondrial unspecific channels. A UCP has been described in Yarrowia lipolytica. Non-pumping alternative oxidoreductases exist in the branched respiratory chain from Y. lipolytica. The variety of energy dissipating systems in eukaryote species is probably designed to control ROS production in the different environments where each species lives.